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*Published in:*  
Danish Medical Bulletin (Online)

*Publication date:*  
2014

*Document Version*  
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

*Citation (APA):*  
Petersen, K. B., Johansen, H. K., Rosthoj, S., Krebs, L., Pinborg, A., & Hedegaard, M. (2014). Increasing prevalence of group B streptococcal infection among pregnant women. *Danish Medical Bulletin (Online)*, 61(9), [A4908].

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# Increasing prevalence of group B streptococcal infection among pregnant women

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## ABSTRACT

**INTRODUCTION:** Group B streptococci (GBS) can cause preterm delivery for women and sepsis and meningitis in infants younger than 90 days of age. The present retrospective cohort study determines the trend over time in the rates of GBS and in demographic risk factors for GBS among pregnant women delivering at Rigshospitalet (RH).

**MATERIAL AND METHODS:** In the period from 2002 to 2010, a total of 33,616 women gave birth at the RH. Our cohort was defined as 16,587 (49%) women examined by 24,724 cultures. All microbiological requisitions from the Department of Obstetrics at RH were extracted from the Clinical Microbiology Database. Maternal data were obtained from a local database at the RH.

**RESULTS:** In our cohort, a total of 638 (3.8%) women were diagnosed with GBS, 517 (81%) from urine, 92 (14%) from vaginal swabs and 29 (5%) from both. The overall rate of women colonised with GBS rose from 3.3% in 2002 to 5.1% in 2010 ( $p < 0.0001$ ). A total of 48 infants had early-onset group B streptococcus (EOGBS), 1.4 per 1,000 neonates in the general population and 7.8 per 1,000 among women with GBS ( $p < 0.0001$ ).

**CONCLUSION:** We found a low GBS colonisation rate in our pregnant cohort, but the rate followed an increasing trend over the study period. GBS during pregnancy was associated with a low birth weight and preterm delivery. More research on preventive measures is needed, but updated guidelines, screening and intrapartum antibiotic prophylaxis continue to be the cornerstones of EOGBS disease prevention.

**FUNDING:** not relevant.

**TRIAL REGISTRATION:** not relevant.

Group B streptococci (GBS) can cause preterm delivery for women and sepsis and meningitis in infants younger than 90 days of age. Maternal colonisation with GBS in the genitourinary tract is the primary risk factor [1]. Colonisation is generally asymptomatic and only occasionally associated with urinary tract infection [2]. Early-onset GBS (EOGBS) < 1 week of age is the leading cause of serious neonatal infections in the UK and the US [2, 3]. The incidence of EOGBS disease has declined over the past 15 years, from 1.7 cases per 1,000 live births in the early 1990s to 0.34-0.37 cases per 1,000 live births in recent years [4]. In the 1970s, the fatality rate among

babies with EOGBS was approximately 50% in the US, but the rate has declined to 4-6% in recent years, primarily owing to advances in neonatal care [1, 3, 5].

Despite improvements in medical care, GBS remains an important cause of perinatal morbidity and mortality. The prevalence of diseases caused by invasive beta-haemolytic streptococci is rising in Denmark [6]. The reported colonisation rate among Danish women in obstetrical cohorts fluctuates from 8% to 36%, despite the fact that the cohorts were remarkably homogeneous [7, 8]. In a recent review, the GBS vaginal colonisation rates in Europe ranged from 6.5% to 36%, similar to the US where 10-30% of pregnant women are colonised [5, 9]. There are competing strategies for the prevention of EOGBS disease. In the US, universal culture-based screening has yielded a substantial decline in disease incidence since it was recommended in 2002 [10]. At Rigshospitalet (RH), intrapartum antibiotic prophylaxis (IAP) has been administered based on a risk-factor-based approach since 2004. IAP is administered to women with preterm delivery < 37 gestational weeks, intrapartum temperature > 38° C, amniotic membrane rupture ≥ 18 hours, GBS bacteriuria during any trimester of the current pregnancy, or a previous infant with invasive GBS disease [11].

This study describes the incidence of mothers with a positive GBS culture during pregnancy who gave birth at RH, and it describes the association between the incidence and neonatal GBS morbidity and mortality in the 2002-2010 period.

## MATERIAL AND METHODS

### Definition of the cohort

We conducted a retrospective cohort study on all deliveries at the RH from 2002 to 2010. The cohort included 33,616 women. Maternal data were obtained from a local RH database by using the Danish Personal Identification number (CPR). The obstetrical and neonatal data were obtained from a local obstetrical database (OD) covering RH and Hvidovre Hospital. We extracted all microbiological requisitions from the Clinical Microbiology Database (MADS) at the RH. If a woman had a minimum of one positive bacterial sample with GBS, she was defined as GBS-positive. The following data were extracted for each woman: date and gestational age at birth, birth

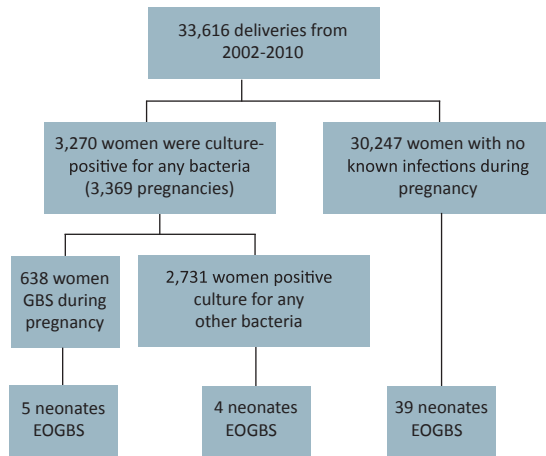
## ORIGINAL ARTICLE

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Dan Med J  
2014;61(9):A4908

FIGURE 1

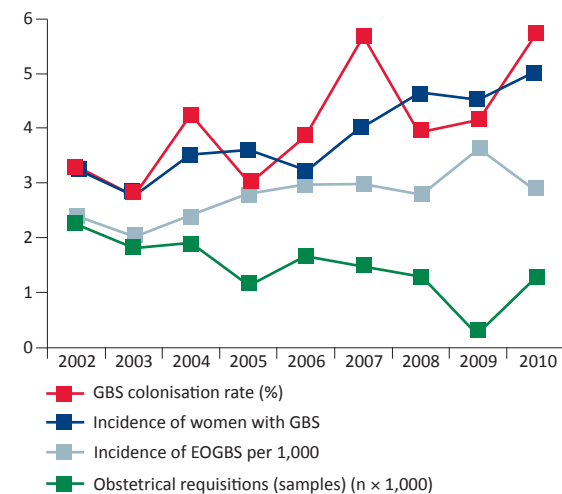
Patient flow chart.



EOGBS = early-onset Group B streptococci < 1 week of age; GBS = group B streptococci.

FIGURE 2

Group B streptococci colonisation rate, incidence of women with Group B streptococci during pregnancy and preterm delivery, Obstetrical requisitions and incidence of early-onset Group B streptococci per 1,000 neonates among women giving birth at Rigshospitalet from 2002 to 2010.



EOGBS = early-onset group B streptococci < 1 week of age; GBS = group B streptococci.

weight, pH in the umbilical cord, Apgar score, mode of delivery, maternal age at delivery, parity, maternal body mass index (BMI) and smoking habits. The study population with in- and exclusions is described in **Figure 1**.

To identify the neonates with a positive bacterial culture for GBS, we used MADS at the RH. We defined the search to include all neonates aged 0-90 days with at least one positive GBS bacterial culture (blood, cerebro-

spinal fluid and swabs from the trachea) and admission to the neonatal ward due to septicaemia. From 2002 to 2010, we identified 48 neonates matching these criteria. We identified their mothers and data regarding delivery from the OD.

### Microbiological cultures

The samples were collected according to departmental guidelines. Indications for urine samples were premature contractions, preterm pre-labour rupture of membranes (PPROM), symptoms of urinary tract infections and former preterm delivery, etc. Indications for vaginal swabs were PPROM or possible infections.

The urine samples were collected as clean catch urine. If the transportation time to the laboratory exceeded two hours, the samples were stored in refrigerators.

In brief, all urine samples were handled as follows throughout the period: microscopy was performed on all urine samples and 100 µl were cultured on 5% Danish blood agar (DBA). In case of bacteria visualised by microscopy, we added a "blue plate" (modified Conradi Drigalski's medium) selective for Gram-negative rods, a tellur plate (State Serum Institute, Copenhagen, Denmark), a blood agar resistance plate and a chromID CPS plate (BioMerieux) specific for GBS. Bacteria with different susceptibility patterns and different colony morphologies were chosen and identified according to internal laboratory manuals.

The vaginal swabs were collected by swabbing the lower vagina and were stored in transportation media and handled as mentioned above.

Vaginal swabs were cultured on 5% DBA, a "blue plate", a Chrom agar plate for *Candida* species, a Gonococci plate and a *Gardnerella* plate. All cultures were incubated for two days. The DBA was incubated in CO<sub>2</sub> to promote growth of GBS.

### Statistics

The Pearson  $\chi^2$ -test was applied to compare frequencies. Differences in frequencies were summarised by odds ratios (OR). Confidence intervals (CI) for proportions were calculated based on the clog-log transform. Univariable logistic regression analyses were performed to examine the associations between calendar year and risk of positive GBS bacterial culture and adverse neonatal outcomes.

### Ethics approval

The study was approved by the Danish National Data Protection Agency. Reference number: 41-0569. (Date: 1 March 2011).

*Trial registration:* not relevant.



TABLE 1

Examination rate, group B streptococci colonisation rate and incidence of early-onset group B streptococci from 2002 to 2010.

	2002	2003	2004	2005	2006	2007	2008	2009	2010	Total
Deliveries in Denmark <sup>a</sup> , n	62,315	62,812	62,874	62,545	63,201	62,535	63,327	61,131	61,445	562,185
Deliveries at RH, n	3,074	3,303	3,220	3,316	3,729	3,954	3,783	3,864	5,373	33,616
Examination rate, %	52	44	49	56	53	50	51	60	37	49
Women with a positive GBS culture during pregnancy, n	53	40	55	58	63	78	89	100	102	638
GBS colonisation rate, %	3.3	2.8	3.5	3.2	3.2	4.0	4.6	4.3	5.1	3.9
National incidence of preterm deliveries <sup>a</sup> , %	6.2	6.3	6.3	6.2	6.2	6.4	6.0	5.8	5.8	6.1
Incidence of preterm deliveries RH, %	10.9	10.7	11.0	11.3	10.7	10.4	10.8	11.5	8.1	10.5
Women with GBS during pregnancy and preterm delivery, n	11	10	15	11	15	23	16	18	25	144
Incidence of women with preterm delivery and GBS during pregnancy, %	3.3	2.9	4.2	2.9	3.8	5.6	3.9	4.0	5.7	4.1
Neonates with EOGBS, n	7	6	6	4	6	6	5	1	7	48
Incidence of EOGBS per 1,000 neonates, n	2.3	1.8	1.9	1.2	1.6	1.5	1.3	0.3	1.3	1.4

EOGBS = early-onset group B streptococci < 1 week of age; GBS = group B streptococci; RH = Rigshospitalet.

a) Source: Danish Health and Medicines Authority.

## RESULTS

### Colonisation and infections with group B streptococci

From 2002 to 2010, a total of 33,616 women delivered at the RH. Of these, 16,587 (49%) were examined with a vaginal swab or urine culture or both. Overall, 3,270 of the 16,587 women (19.7%) were culture-positive for any bacteria.

We identified 5,518 positive cultures from 3,270 women and 3,369 pregnancies. The main sample categories of the positive cultures were 3,803 urine tests (67%) and 1,242 vaginal swabs (22%). The proportion of pregnancies with at least one examination for GBS ranged between 37% and 60% of the annual birth cohort during the study period (Table 1).

We identified 638 women with GBS (510 urine samples, 85 vaginal swabs, 29 with a culture-positive urine and vaginal swab, 14 from other categories). The overall rate of women colonised by GBS in our cohort was 3.9% (638/16,587; 95% CI: 3.6-4.1%). The GBS colonisation rate in our cohort varied from 2.8% to 5.1% (Figure 2 and Table 1). Our data demonstrate a significant increase in GBS-positive samples in our cohort from 3.3% (53/1,598) in 2002 to 5.1% in 2010 (102/1,988) (OR = 1.07; 95% CI: 1.04-1.11;  $p < 0.0001$  per year). The overall rate of GBS-positive samples increased significantly from 13% (53/418) in 2002 to 29% (102/357) in 2010 (OR = 1.13; 95% CI: 1.09-1.17;  $p < 0.0001$  per year). We found a similar, significant increase in both GBS-positive vaginal swabs (OR = 1.21; 95% CI: 1.11-1.31;  $p < 0.0001$  per year) and GBS in urine (OR = 1.12; 95% CI: 1.08-1.16;  $p < 0.0001$  per year).

In the youngest and oldest maternal age groups, we found a tendency towards an increased risk of GBS. Furthermore, we found an increased risk in multiparity (Table 2).

### Early-onset group B streptococci

We identified 48 neonates with EOGBS. The incidence of EOGBS was five in 638 (0.7%) infants born by mothers with a positive GBS culture compared with 39 (0.2%) in 30,247 in the women with no diagnosed infection during pregnancy, and four infants (0.1%) born by mothers with culture-positive samples, but with other bacteria than GBS. Women with a positive culture for GBS during pregnancy had an increased risk of giving birth to an infant with GBS septicemia compared with women with a negative GBS culture (OR = 6.05; 95% CI: 2.39-15.32). We observed no difference in the risk of delivery of a child with GBS septicemia between mothers with a negative GBS culture sample and mothers with a positive culture with bacteria other than GBS. There were no neonatal deaths due to GBS among the 48 neonates. Of the neonates with GBS culture-positive mothers, three out of five received intrapartum antibiotic prophylaxis (IAP) in relation to a caesarean section. In total, 21 mothers to the 48 neonates with EOGBS received IAP.

### Duration of labour, prelabour rupture of membranes/premature prelabour rupture of membranes and obstetrical outcomes

We examined the duration from rupture of membranes (ROM) to delivery among the 48 neonates. This information was available for 40 cases. Our data showed that only 5% (two out of 40 neonates) (95% CI: 1-15%) had a prolonged ROM (> 18 hours), and none exceeded 22 hours.

There was no trend in either pre-labour ROM (PROM) or premature pre-labour ROM (PPROM) over time in our cohort. But in the women with GBS, we found 4.2% (27/638; 95% CI: 2.9-6.0%) with PPROM compared with 2.9% (940/32,978; 95% CI: 2.7-3.0%) in

TABLE 2

Demographic data, risk factors and obstetrical outcomes comparing our group B streptococci culture-positive cohort of 638 women and all other deliveries from 2002 to 2010.

Demographic data	GBS-positive, n (%)	All other deliveries, n (%)	OR (95% CI)	p-value <sup>a</sup>
<b>Risk factors</b>				
Maternal age, yrs				0.0240
15-19	7 (4.1)	163 (95.9)	2.44 (1.13-5.25)	
20-24	43 (2.3)	1,866 (97.7)	1.31 (0.94-1.81)	
25-29	160 (1.8)	8,794 (98.2)	1.03 (0.84-1.26)	
30-34	236 (1.7)	13,369 (98.3)	Ref.	
35-39	158 (2.2)	7,015 (97.8)	1.28 (1.04-1.56)	
≥ 40	34 (2.3)	1,453 (97.7)	1.37 (0.92-1.91)	
Data missing	0 (-)	318 (-)	-	
Total	638	32,978	-	
Parity, no. of previous births				0.0009
0	306 (1.7)	17,885 (98.3)	Ref.	
1	213 (2.0)	10,600 (98.0)	1.17 (0.98-1.40)	
2	82 (2.6)	3,068 (97.4)	1.56 (1.22-2.00)	
≥ 3	37 (2.5)	1,425 (97.5)	1.52 (1.07-2.14)	
Data missing	0 (-)	0 (-)	-	
Total	638	32,978	-	
<b>Outcomes</b>				
Gestational age at birth, weeks				< 0.0001
< 28	28 (4.5)	763 (2.2)	-	
28-31	30 (4.8)	707 (2.1)	-	
32-36	86 (13.7)	2,890 (8.4)	-	
37-41	412 (65.5)	28,482 (82.5)	-	
≥ 42	73 (11.6)	1,704 (4.9)	-	
Data missing	9 (-)	7 (-)	-	
Total	638	34,553 <sup>b</sup>	-	
Birth weight, g				< 0.0001
≤ 999	25 (4.0)	779 (2.2)	-	
1,000-1,999	59 (9.4)	1,257 (3.7)	-	
2,000-2,999	158 (25.2)	6,205 (18.1)	-	
3,000-3,999	344 (54.7)	21,525 (62.4)	-	
4,000-4,499	33 (5.3)	3,934 (11.4)	-	
≥ 4,500	9 (1.4)	773 (2.2)	-	
Data missing	10 (-)	56 (-)	-	
Total	613	34,529 <sup>b</sup>	-	
Mode of delivery				< 0.0001
Vaginal (non-instrumental)	234 (36.7)	21,543 (65.3)	-	
Vaginal (instrumental)	77 (12.1)	2,802 (8.5)	-	
Elective caesarean section	141 (22.1)	4,131 (12.5)	-	
Acute caesarean section	186 (29.1)	4,502 (13.7)	-	
Data missing	0 (-)	0 (-)	-	
Total	638	32,978	-	

CI = confidence interval; GBS = group B streptococci; OR = odds ratio.

a) Pearson  $\chi^2$ -test.

b) Due to multiple pregnancies, the number of babies exceeds the number of births (39 GBS multiple pregnancies).

95% CI: 1.95-3.11) and of instrumental vaginal, elective caesarean section and emergency caesarean section (OR = 3.25; 95% CI: 2.76-3.83) (Table 2). The incidence of GBS among the women with preterm delivery increased from 3.3% (95% CI: 1.7-5.6%) in 2002 to 5.7% (95% CI: 3.8-8.2%) in 2010 (Table 1). In the trend analysis, we found that the risk of a GBS-positive culture tended to increase over time (OR = 1.07; 95% CI: 1.00-1.14;  $p$  = 0.048).

## DISCUSSION

We found a significant increase in the number of GBS-positive samples in our cohort over time, particularly among the young and elderly women and the multiparous. We found an increased risk of preterm delivery among women colonised with GBS during pregnancy and, furthermore, a six-fold increased risk of delivery of a child with EOGBS in the perinatal period. The overall incidence of EOGBS was 1.4 cases per 1,000 live births, which is substantially higher than in other countries [12]. The national incidence of EOGBS was 0.6 cases per 1,000 live births in 1995, but 0.2 cases per 1,000 live births in 2002 [13]. The RH is a referral centre for preterm deliveries; and selection bias may thus explain the higher EOGBS rate at the RH. Over time we found a decrease in the incidence of EOGBS from 2.27 cases per 1,000 live births in 2002 to 1.30 cases per 1,000 live births in 2010. This decrease could be caused by the introduction of IAP from 2004 [11] and further by a hospital fusion in 2009, which increased the proportion of low risk births at our unit. There are conflicting reports about GBS as a risk factor for preterm delivery or preterm rupture of membranes [14-17]. The increased risk of preterm birth in mothers with a positive GBS culture in our study could be due to more frequent testing of women with preterm or imminent preterm birth.

Our data showed a rising trend in the prevalence of GBS-positive samples in women tested at the RH and an increase in the incidence of GBS in mothers with preterm deliveries. This was not followed by an increase in associated complications such as preterm delivery and EOGBS. Interpretation of the results should take into account that the examination rate varied from 37% to 60% during the study period and that there were no strict criteria for performing the tests. Thus, we cannot document a rising prevalence of GBS, but our results raise a suspicion that warrants further consideration. The colonisation rate was low compared with earlier Danish studies, which could be due to the fact that our cultures were performed on indications and not as a general screening. By screening all pregnant women with vaginal swabs we would be able to find the asymptomatic, colonised women and hence would observe a higher colonisation rate [7, 13, 18].

the background population of 32,978 deliveries (OR = 1.51; 95% CI: 1.02-2.23;  $p$  = 0.039).

Colonisation with GBS during pregnancy increased the risk of preterm delivery < 37 weeks (OR = 2.06; 95% CI: 1.70-2.48), low birth weight < 2,000 g (OR = 2.46;





Haemolytic group B streptococci.

GBS bacteriuria in pregnant women is a marker for heavy colonisation and an increased risk of EOGBS [10]. This emphasises the importance of detecting GBS in pregnancy in order to be able to devise a relevant IAP strategy. Whether or not to screen all pregnant women remains a controversial issue, but since the Centre for Disease Control (CDC) and the American College of Obstetricians and Gynecologists (ACOG) introduced a national screening programme in 1996, the prevalence of EOGBS has declined substantially in the US [10]. By 2008, the incidence diminished from 1.5-1.7 cases per 1,000 live births to 0.34, i.e. a decrease of more than 80% [4]. Routine screening has now been introduced in Australia, Spain, Italy, Belgium and Germany [19]. The efficacy of a vaginal screening regime significantly reduces the risk of neonatal EOGBS (RR 0.5, 95% CI (0.4-0.6)) [5].

A total of 19% of the babies with EOGBS in this study were born prematurely (28-36 gestational week) and 49% born very prematurely (< 28 gestational week). The fact that the mortality is higher among preterm infants with EOGBS, with case-fatality rates of approximately 20% and reaching 30% among those  $\leq$  33 gestation weeks compared with 2-3% among full-term infants underlines the severity of the increase in GBS colonisation among pregnant women [1]. There were no deaths in our neonatal GBS cohort, which could be owed to the highly specialised and advanced neonatal care on the RH.

A recent Cochrane review concluded that most studies conducted on GBS colonisation are biased due to methodological weaknesses and that there is a lack of evidence from well-designed and well-conducted trials to recommend IAP to reduce neonatal EOGBS and that the effectiveness of IAP should be studied in RCTs [20].

## CONCLUSION

GBS colonisation among pregnant women remains the leading cause of serious neonatal infections with EOGBS and attention to this condition is warranted. Changing

the antenatal care recommendations in Denmark from the existing risk-factor-based IAP to a national screening procedure and hence a substantial increase in the use of IAP could be a cost-full and time-consuming process, and neither this study nor the available literature in the field provide sufficient grounds for changing the present practices, and further randomised studies are therefore needed.

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**ACCEPTED:** 3 July 2014

**CONFLICTS OF INTEREST:** none. Disclosure forms provided by the authors are available with the full text of this article at [www.danmedj.dk](http://www.danmedj.dk).

**ACKNOWLEDGEMENTS:** We would like to thank *Bonnie Horsted Eriksen* for data extraction from the Microbiology Database, *Susanne Mårtensson* for data extraction from the local database at Rigshospitalet, and *Carsten Nickelsen* for data extraction from the Obstetrical Database.

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